Transformation into Rhizobium strains

We pursued the transformation of plasmid pKT230 into the strains for expression of the beta-homologue oligonucleotide annealing protein for MAGE. According to the table below, electroporation was successful in *R. tropici CIAT 899* for all attempts. Growth on negative controls in *S. meliloti* strains in attempt 1 was suspected to be contamination. No growth in *S. meliloti* strains in attempt 2 was attributed to low transformation efficiency. To increase transformation efficiency, electroporation voltage was increased to 2kV applying 4 pulses in 15-second intervals for each electroporation in attempt 3, as opposed to a single 1.8kV pulse in attempts 1 and 2. Additionally, kanamycin concentration was increased for better selection but lawns grew on all strains, indicating that the OD_{600nm} of the cells used for transformation may be too high or the TSB media may be to rich to select for cells using kanamycin. TSB has a higher concentration of phosphate compounds than LB or YMB, which may disable the effectiveness of the kanamycin antibiotic. Because electroporation of pKT230 into *R. tropici CIAT 899* was most successful, it was concluded that *R. tropici CIAT 899* is a suitable candidate for MAGE. In latter experiments, *S. meliloti 370* was also found to be reliably electrocompetent (see Promotoer-Citrine Fluorescence Data); thus *S. meliloti 370* was also deemed a suitable candidate for MAGE.

Four attempts of electroporation plasmid pKT230 into Rhizobium strains and selection using Kanamycin TSB plates.

Attempt	***Organism***	***Selection***	***Growth on Neg. Control***	***Growth on Plasmid Pos.***
1	CIAT 899	30 µg/mL Kan	NO	YES
	Sm356	30 µg/mL Kan	YES	YES
	Sm370	30 µg/mL Kan	YES	YES
	Sm371	30 µg/mL Kan	YES	YES
2	CIAT 899	30 µg/mL Kan	NO	YES
	Sm356	30 µg/mL Kan	NO	NO

	Sm370	30 µg/mL Kan	NO	NO
	Sm371	30 µg/mL Kan	NO	NO
3	CIAT 899	180 µg/mL Kan	NO	YES
	Sm371	180 µg/mL Kan	YES	YES
4	CIAT 899	30 µg/mL Kan	NO	YES
	Sm371	180 µg/mL Kan	YES	YES